

## ANATOMICAL AND MORPHOLOGICAL CHANGES IN *PINUS HELDREICHII* CHRIST ALONG AN ALTITUDINAL GRADIENT IN PIRIN MOUNTAINS

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**Abstract.** The paper presents the results of a study on the variation of morphological and anatomical traits of needles and tree ring width in several sites of *Pinus heldreichii* CHRIST in Pirin Mountains, Bulgaria. The tree ring width decreased with the increase of altitude. The summer drought and insufficient rainfalls had negative effect on the tree growth, while August precipitation affected it positively, thus showing that the species reacts to the climate variables in a complex way. The needle traits were not dependent on the altitude, but showed some relationship to exposition. Tree ring chronologies proved to be promising for the construction of reliable and long proxy climate records for the region.

**Keywords:** *Pinus heldreichii*, tree ring chronologies, needle traits, site conditions.

**Rezumat. Schimbări anatomice și morfologice la *Pinus heldreichii* CHRIST de-a lungul unui gradient altitudinal din Munții Pirin.** Articolul prezintă rezultatele unui studiu privind variația caracteristicilor morfologice și anatomice ale acelor și lățimii inelelor copacilor în câteva areale cu *Pinus heldreichii* CHRIST din Munții Pirin, Bulgaria. Lățimea inelelor descrește pe măsură ce altitudinea crește. Seceta înregistrată vara și cantitățile insuficiente de precipitații au avut un efect negativ asupra creșterii copacilor, în timp ce precipitațiile înregistrate în august au avut o influență pozitivă, indicând astfel faptul că speciile reacționează la variabilele climatice într-un mod complex. Caracteristicile acelor nu depind de altitudine, dar au indicat o oarecare legătură cu expoziția versanților. Cronologiile inelelor copacilor s-au dovedit promițătoare pentru determinarea înregistrărilor climatice proxy viabile și de lungă durată în regiune.

**Cuvinte cheie:** *Pinus heldreichii*, cronologiile inelelor copacilor, caracteristicile acelor, condițiile site-ului.

### INTRODUCTION

*Pinus heldreichii* CHRIST (*Pinus leucodermis* ANTOINE) is a tertiary relic species occurring in isolated subalpine and timber line locations in some mountains within the Balkan Peninsula and Southern Italy (BARBERO et al., 1998). In Bulgaria, it grows only in the Pirin and Slavyanka Mountains on soils formed on marble and limestone bedrocks. Due to the small overall territory, the species is under strict protection and all of its locations are included in protected territories – nature reserves and the Pirin National Park. Hardly accessible steep slopes helped the conservation of numerous forests in pristine state and the local treeline is found at the expected altitude that coincides with the 10°C July isotherm (DAKOV et al., 1980). This provides the chance to conduct studies in ecosystems that evolved under small or without any human influence. Although *Pinus heldreichii* CHRIST is of high conservational value, still many topics related to its habitats are not well studied. Among them are typical forest structures, regeneration, morphological and genetic variation. Research was mostly locally based and focused on single parameters (GUDESKI et al., 1975; YURUKOV et al., 2005; PANAYOTOV & ŞESAN, 2007; TODARO et al., 2007; GUERRIERI et al., 2008; PANAYOTOV et al., 2010). Therefore further studies are needed to fill in these gaps. Moreover, besides pure conservational research needs the species additionally provides high potential for proxy climate studies due to its longevity. Initial research in this direction (PANAYOTOV & YURUKOV, 2008; TODARO et al., 2007; PANAYOTOV et al., 2010) demonstrated the possibilities to construct almost millennia-long highly replicated tree ring series.

Here, we aim at studying the variation of tree ring and leaf morphology along altitudinal gradient within one valley. Additionally we compare the influence of exposure on these parameters.

### MATERIAL AND METHODS

The study area is situated in the Banderitza valley in the Pirin Mountains, Bulgaria, 41°45' N, 23°26' E (Fig. 1). We selected three study sites at different elevations for collecting tree ring cores, on the eastern slope of Vihren peak and one on the north-western slope of Todorka peak (Table 1). For leaf morphology we additionally collected samples from two more locations, one on Vihren slope and the other on Todorka slope.

Table 1. Position of study plots.  
Tabel 1. Poziția arealelor studiate.

Site name	Altitude	Exposure	Number of tree ring cores	Number of studied leaves
1800-W	1800	NW	-	50
1950-W	1950	NW	17	50
1750-E	1750	E	15	50
1900-E	1900	E	29	50
1950-E	1950	E	-	50
2000-E	2000	E	-	50
2100-E	2100	E	55	-

The slopes are steep, with an inclination of 20-50° and covered mostly with thin Rendzic Leptosols and Regosols formed on marble bedrock. Forests are pure *Pinus heldreichii* CHRIST at higher elevations and mixed with *Picea abies*, *Pinus sylvestris*, and the Balkan endemic *Pinus peuce* at lower elevations (Table 2). Data for forest structure were collected by setting rectangular 0.2 ha plots (40x50 m).

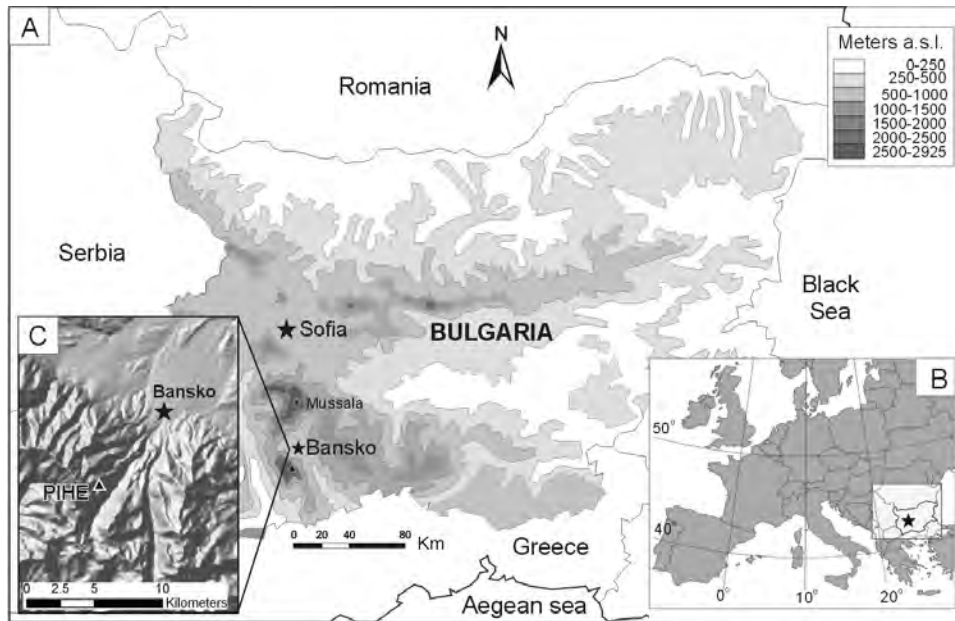


Figure 1. Geographic position of the study area.  
 Figura 1. Poziția geografică a zonei studiate.

Tree ring cores were collected with increment borer at breast height (1.3 m) from 15 to 55 dominant trees per site that were not affected by avalanches or rock-falls. They were mounted on wooden boards, air-dried and sanded. Tree ring widths were measured in the dendrochronology laboratory at the University of Forestry in Sofia following standard procedures with precision of up to 0.001 cm. Obtained tree ring width series were cross-dated with the use of visual clues (STOKES & SMILEY, 1968) and the computer program COFECHA (HOLMES, 1983). Then the data were standardized with the software package ARSTAN (COOK, 1985) using exponential functions. In cases with higher tree ring variation, which was typical mostly for the lower altitude sites, we also used cubic splines to perform the standardization. The final chronologies were composed by calculating bi-weighted robust means of annual ring widths. This, as well as the calculation of standard descriptive parameters was performed with the ARTSAN software. The highest altitude chronology (2100-E) was previously published (PANAYOTOV et al., 2010) and thus we compare newer data to it.

Table 2. Forest structure data for the studied sites.  
 Tabel 2. Date privind structura pădurii în site-urile studiate.

Sample plot	Slope	Tree species, % of trees with DBH>6				Number of trees with DBH>6 per ha
		PIHE	PIPE	PISY	PCAB	
1750-E	30	69	-	1	30	997
1800-W	20	28	17	-	53	725
1900-E	45	91	-	-	9	516

Abbreviations: PIHE – *Pinus heldreichii* CHRIST., PIPE – *Pinus peuce* GRISEB., PISY – *Pinus sylvestris* L., PCAB – *Picea abies* (L.) KARST.

For the study of leaf morphology we collected 5 two-year old needles from the lower southern parts of the crowns of 10 trees at each site (e.g. 50 needles per site). We measured the length of the needles (Lf) with accuracy of up to 1 mm, the width (W) and thickness (D) with accuracy of up to 0.1 mm. Anatomical features were observed and recorded on micro-sections obtained from the mid-length of the leaf. We present the results for the number of resin ducts.

The analysis of the climate-tree ring growth relationship was performed with DENDROCLIM2002 software (BIONDI & WAIKUL, 2004) using average monthly temperatures and precipitation sums for the months from June of the year prior to growth to September of the current year. The software uses 1000 bootstrapped samples to compute response and correlation coefficients, and to test their significance at the 0.05 level. Median correlation and response coefficients are deemed significant if they exceed, in absolute value, half the difference between the 97.5-th quantile and the 2.5-th quantile of the 1000 estimates (BIONDI & WAIKUL, 2004). The climate data for the analysis was obtained from Bansko station (936 m a.s.l.) It is located at the foot of the mountain, 10 km away of the study area and provides continuous records for more than 70 years (since 1931). Vihren chalet climate station data was used to describe the climate in the study region. It is in the study valley, but it has been functioning for only 25 years. The climate in the

region is typically mountainous, with strong influence of the Mediterranean air masses. The mean annual temperature (Vihren chalet climate station, 1970 m a.s.l.) is 3.5°C. It ranges from a mean monthly temperature of -4.7°C in January to +12.2°C in August. The annual temperature at the timber line, obtained by extrapolation, is 1.6°C; the highest average monthly temperature is 10.2°C. This coincides with the expected values of nearly 10°C in the warmest month at the timber line (TRANQUILLINI, 1979; DAKOV et al., 1980). The annual precipitation amounts to 1,378 mm, with a maximum in autumn and winter. Deep snow covers are characteristic for the region. It is worth mentioning that the absolute maximum snow depth for Bulgaria (472 cm) was recorded at Vihren chalet station. At the same time, the summer precipitation minimum combined with shallow soil profiles on steep rocky sites might cause local drought conditions on sites with eastern and southern exposure (PANAYOTOV et al., 2010).

## RESULTS AND DISCUSSIONS

Tree ring width was the highest at the lowest altitude site (Table 3). This was valid for minimum, maximum and average values. The trees that were growing on the shady north-western slope had lower value of average tree ring width than the similar altitude on the sun-exposed slope. However, this pattern was not true for the minimum and maximum values. Such finding is a clue, that at least some of the trees on the shady slope grew better than on the sun-exposed. Yet, for the majority the tendency was reverse. The trees in the highest altitude (i.e. site 2100-E) had the lowest average, minimum and maximum tree ring widths. Compared to the widths of the trees growing 300 m lower (1750-E), these differences were more than double. The average and minimum values might be influenced by the age differences, because most of the trees in the highest altitude site are much older than those at lower altitudes. It is known that with aging light-demanding coniferous species decrease gradually their radial growth until a “plateau” is reached in which the trees produce more or less similarly wide tree rings (FRITTS, 1976). This gradual decrease phase lasts 100-150 years at *Pinus heldreichii* CHRIST trees (PANAYOTOV, 2007) and thus if the tree ring width of old trees is compared to the one of young there is a risk that the average values are influenced to a greater extent by the length of the cores. Yet, the differences detected in the maximum values were dependent much more on the growth of the specific individual than on the length of the cores. Thus, they can be considered a clear sign of lower radial growth at higher elevations. This is expected having in mind that in high mountains radial growth is generally influenced by the temperature regime and normally decreases with the increase of altitude and decrease of temperatures (FRITTS, 1976; SCHWEINGRUBER, 1996).

More interesting tendencies were observed for the parameter “Sensitivity”, which expresses the year-to-year variations in tree ring width (COOK, 1985). It is a good measure of the fitness of chronologies to represent the climate variation. The higher this parameter is, the stronger the influence of climate is considered to be. The sensitivity was highest at the chronology 2100-E. The next highest value was that for the chronology 1900-W, closely followed by the chronology 1950-E. This tendency is a sign that the lower altitude chronologies have more consistent growth that is limited to a lesser extent by climate extremes. Such a tendency is also revealed if climate-growth correlations are considered.

Table 3. Tree ring chronologies parameters.  
Tabel 3. Parametrii cronologiilor inelelor copacilor.

Chronology name	Max. Length, years	Year span	Series, No.	Mean tree ring width, cm	SD, mean width, cm	Min. tree ring width, cm	Max tree ring width, cm	Sensitivity	Auto-correlation (1 <sup>st</sup> )
1750-E	293	1716-2008	16	0.176	0.09	0.088	0.314	0.194	0.809
1950-E	318	1691-2008	29	0.105	0.06	0.030	0.241	0.205	0.809
1900-W	566	1443-2008	17	0.101	0.04	0.054	0.291	0.206	0.761
2100-E	803	1026-2008	55	0.071	0.03	0.018	0.164	0.218	0.769

The radial growth of the lowest chronology is positively significantly correlated only with the January temperatures (Fig. 2). However, since the trees are in dormancy in that period, such a direct correlation is physiologically unexplainable. Until more precise studies are available, the reason for such consistent correlation can only be hypothesized (PANAYOTOV et al., 2010). Higher correlation coefficients were found for the growth-precipitation relationships. June precipitation of the previous year negatively influenced radial growth, while August precipitation influenced it positively. The negative June precipitation influence can be a direct sign that cold and rainy early-summer period did reduce chances for production of wide tree rings at the lower altitude sites. Such finding is expected for high-mountain locations, where usually trees respond positively to higher summer temperatures (FRITTS, 1976; ESPER et al., 2005; BUNTGEN et al., 2006). Yet it is not in line with the correlation coefficients for the other chronologies, at which June precipitation was not found to correlate with radial growth. In contrast, the highest chronology is positively influenced by current June precipitation. It is also negatively influenced by summer temperatures of both the previous and current growth seasons. Such correlations were also found in previous studies of this species (TODARO et al., 2007; PANAYOTOV et al., 2010), but are in contrast with the expected positive temperature influence for the high mountains.

Yet, a specific feature of our higher altitude sites is the very shallow soil profile. The trees are usually growing on very steep rocky sites. Thus, soils have limited water-holding capacity and rooting is often directly in rock cracks. The “karst” type of terrain also means that available water is easily drained. This, combined with the typical for the region summer precipitation minimum creates conditions for drought-type reactions. PANAYOTOV et al. (2010) demonstrated by analysis of pointer years that in cases of unusually low summer precipitation, especially in the June-July period, timber line *Pinus heldreichii* CHRIST trees produce very narrow tree rings.

The statistically significant correlation coefficients show that the tree ring chronologies might be used for proxy climate reconstructions in case the climate-growth relationships are stable over time. As it has been demonstrated for the chronology 2100-E by PANAYOTOV et al., (2010), the summer correlations are stable. Yet, a difficulty in this term is the “mixed” climate signal. In the cases when more than one climate parameter is correlated with the tree ring growth it is difficult to isolate a single one, on which to base the reconstruction. Therefore further studies to find a more specific climate-signal of just one specific parameter (i.g. summer temperatures) are needed. Initial studies of maximum latewood density of *Pinus heldreichii* CHRIST trees (IVANOVA et al., 2010) demonstrate that it is highly and positively correlated with current August temperatures. This provides the chance to construct long proxy records for the Southern parts of the Balkan Peninsula, which are scarce and limited only for isolated regions (XOPLAKI et al., 2001; LUTERBACHER & XOPLAKI, 2003; POPA & KERN, 2008; PANAYOTOV et al., 2010).

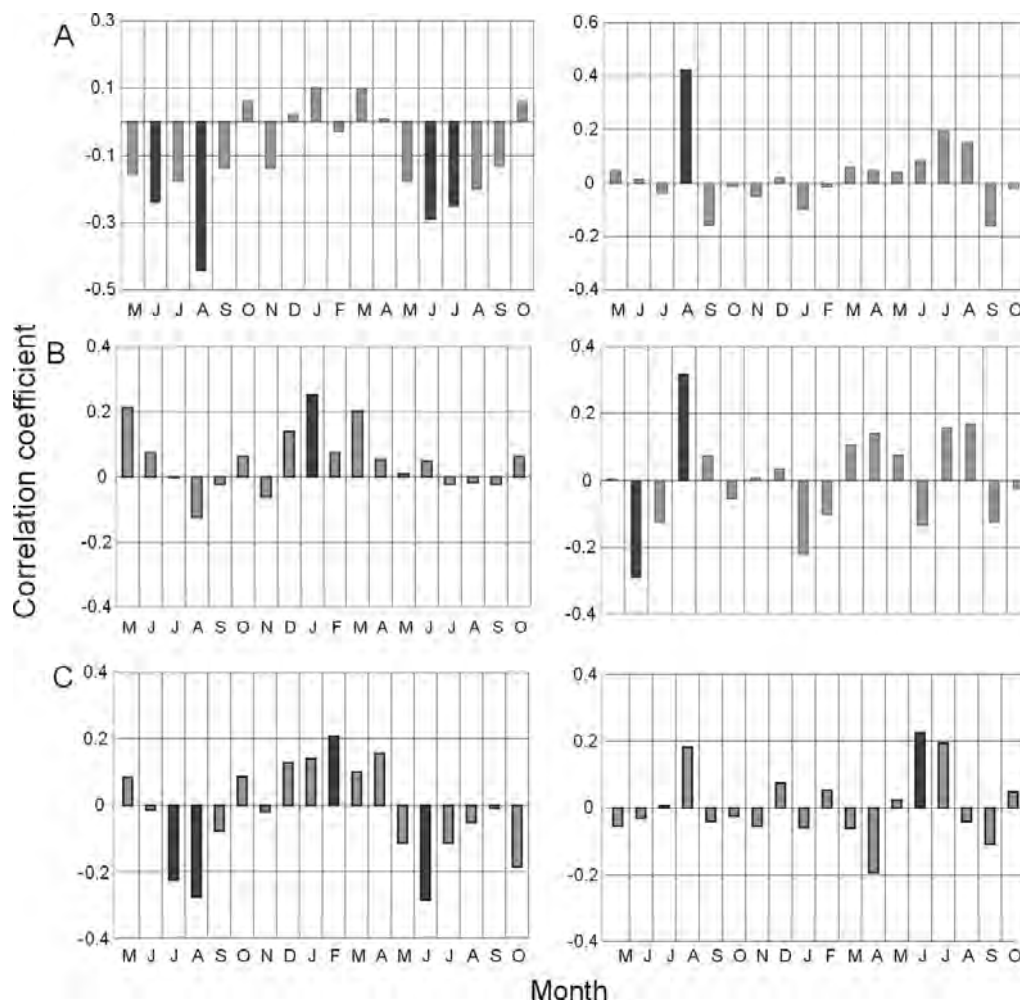


Figure 2. Correlation coefficients for the climate-growth relationships of *P. heldreichii* chronologies 1950-W (A), 1750-E (B) and 2100-E (C). Statistically significant values are marked with black bars.

Figura 2. Coeficienții de corelație pentru relația climat-creștere la *P. heldreichii* chronologies 1950-W (A), 1750-E (B) și 2100-E (C). Valorile semnificative din punct de vedere statistic sunt marcate prin bare negre.

### Leaf morphology

We did not find altitude-dependent variation of the length of leaves (Table 4). The only specific difference is that the maximum values for the shady locations (i.e. sites 1800-W and 1950-W) are lower than those for the sunny slope locations (Fig. 3). The medians are not significantly different. Minimum values also vary in a non-altitude specific manner. They were the highest for site 1950-E and the lowest for site 1900-E. At locations with similar altitudes the leaves of the shady locations are significantly smaller in length than those on the sunny ones (site 1800-W vs 1750-E,  $t = -5.44$ ,  $p < 0.00$ ; site 1950-W vs 1950-E,  $t = -5.70$ ,  $p < 0.00$ ). While this could be a sign, that on shady locations *Pinus*

*heldreichii* CHRIST trees generally produce leaves with smaller length, a sounder conclusion would require more study sites and higher number of samples.

Table 4. Mean values of the studied needle traits.  
Tabel 4. Valorile medii ale caracteristicilor acelor studiate.

	L* mm	W mm	T mm	CW mm	CT mm	D1 μm	NRD n	DRD μm	EHT μm	SR1 n	SR2 n	S1 n	S2 n
1925-W	59.50	1.22	0.71	0.53	0.32	40.52	2.56	38.39	46.92	12.14	6.22	14.02	14.20
1950-W	62.88	1.25	0.72	0.56	0.33	44.26	2.42	41.86	49.59	11.02	5.70	14.94	14.58
1850-E	71.16	1.31	0.75	0.61	0.33	36.44	2.82	46.39	38.92	12.22	6.14	13.86	13.52
1900-E	60.18	1.28	0.75	0.56	0.33	56.92	2.94	49.32	54.92	13.98	6.68	14.46	16.80
1950-E	71.18	1.31	0.76	0.60	0.35	36.18	3.86	46.66	49.85	12.66	6.34	14.94	15.10
2000-E	82.00	1.26	0.72	0.53	0.35	39.90	3.00	39.99	53.32	14.00	6.00	10.00	10.00

\* Legend: L – needle length; W – needle width; T – needle thickness; CW – width of the vascular tissue; CT – thickness of the vascular tissue; D1 – distance between vascular bundles; NRD – number of resin ducts; DRD – mean diameter of resin ducts; EHT – thickness of epidermis and hypoderm; SR1 – number of stomatal rows on the outer surface of the needle; SR2 – number of stomatal rows on the inner surface of the needle; S1 – number of stomata per unit length on the outer surface; S2 – number of stomata per unit length on the inner surface.

\* Legendă: L – lungimea acului; W – lățimea acului; T – grosimea acului; CW – lățimea țesutului vascular; CT – grosimea țesutului vascular; D1 – distanța dintre legăturile vasculare; NRD – numărul de canale de rășină; DRD – diametrul mediu al canalelor de rășină; EHT – grosimea epidermei și hipodermei; SR1 – numărul de rânduri de stomate pe suprafața exterioară a acului; SR2 – numărul de rânduri de stomate pe suprafața interioară a acului; S1 – numărul de stomate pe unitate de lungime pe suprafața exterioară; S2 – număr de stomate pe unitatea de lungime pe suprafața interioară.

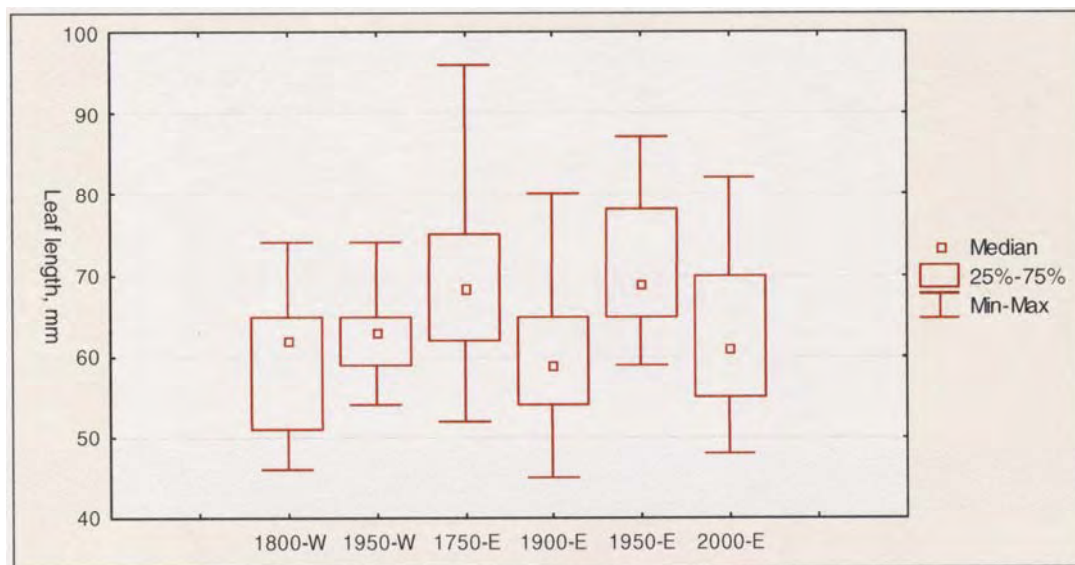


Figure 3. Mean needle lengths.  
Figura 3. Lungimea medie a acelor.

Similarly to leaf length, leaf width (W) was also not found to vary in dependence of the altitude gradient (Fig. 4). No clear pattern of either increase or decrease in the minimum, maximum or average values was observed with the increase of altitude. The minimum values and corresponding average were again found lower for the site located on the shady slope with NW exposure. Yet, as already commented, this could be just a clue that leaves could be generally smaller on shady than sunny locations.

Results for leaf thickness (D) also do not show altitude-dependent differences. Only at the highest locations the average values are lower than at the other locations (data not shown).

Results for leaf thickness (D) also do not show altitude-dependent differences. Only at the highest locations the average values are lower than at the other locations (data not shown). The number of resin ducts was also not found to vary according to the locations. It was higher at the sunny locations at 1950m a.s.l. (Average = 3.86) than the other sites (average varies from 2.50 to 3.00).

The diagram of JENTYS-SZAFEROWA (1950) (Fig. 5) based on the ratio of the trait values at each site to the general mean showed that the most differentiating traits were the distance between vascular bundles, number of resin ducts and stomata per unit length. However, no trend related to the environmental variables was detected.

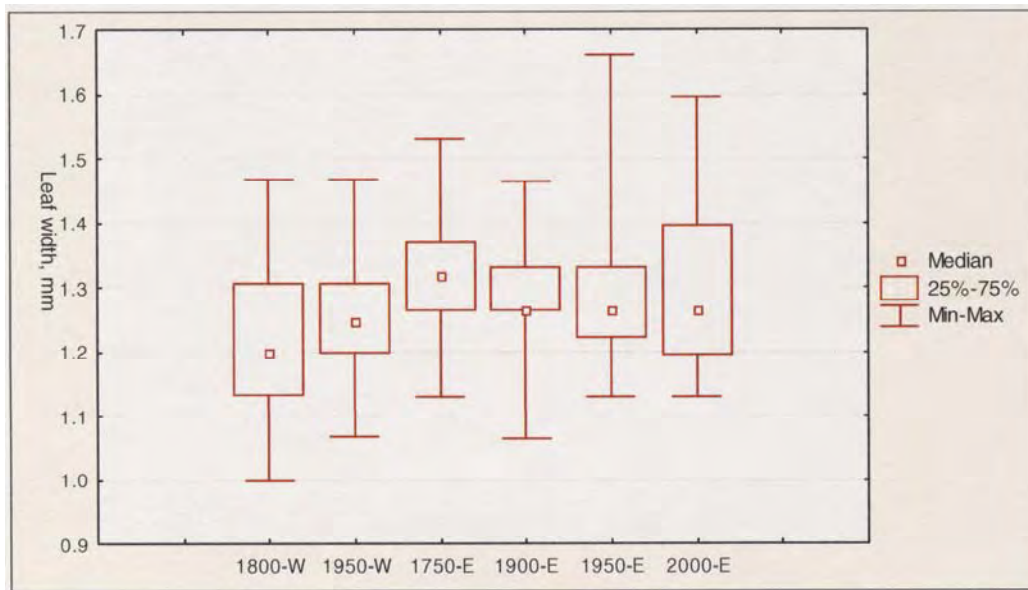


Figure 4. Mean needle width.  
 Figura 4. Lățimea medie a acelor.

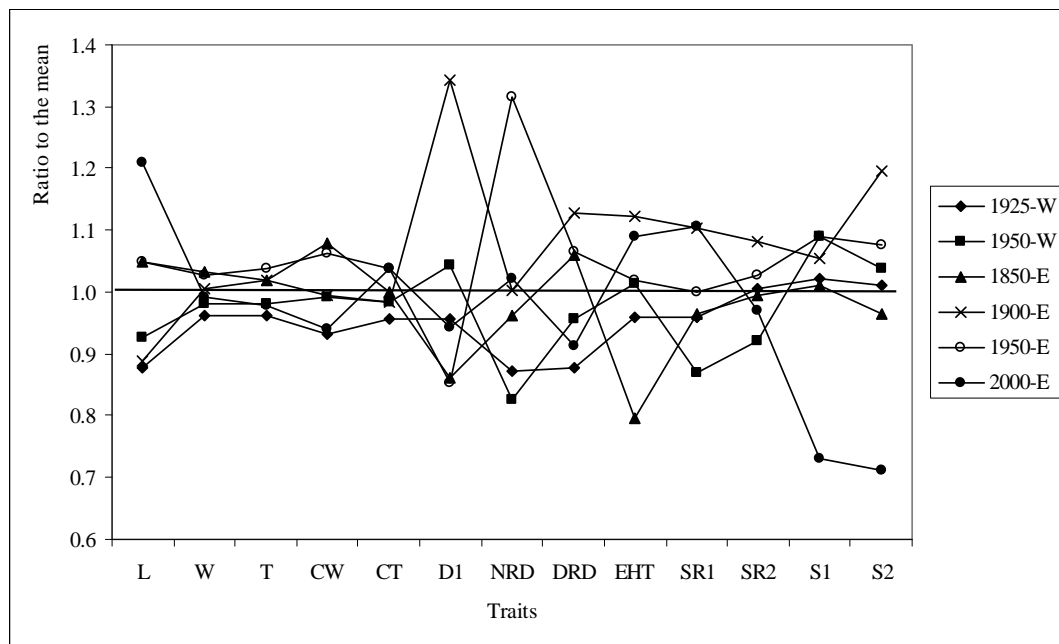


Figure 5. Jentys-Szaferowa diagram showing the most differentiating traits.  
 Figura 5. Diagrama Jentys-Szaferowa care indică cele mai diferite caracteristici.

### CONCLUSIONS

Tree ring widths in *Pinus heldreichii* CHRIST from Pirin were found to decrease with increase of altitude. Climate-growth relationships showed negative influence on radial growth of summer drought-type situations with high temperatures and low precipitation. Increment was positively correlated with August precipitation. This demonstrates that *Pinus heldreichii* CHRIST is responding to climate variability in a contrasting way in comparison with many other high-mountain tree ring chronologies. No altitudinal dependence of leaf morphology (length, width and height) was found. Most of these values were found to be lower at trees growing on shady locations. We consider that for a better differentiation of leaf morphology variation further studies from more locations are needed. Initial results on tree rings chronologies studies give hopes that they can be used for constructing of reliable and long proxy climate records for the Interior Balkan Peninsula.

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